and it also serves as an appropriate and convenient starting point for investigations on protein folding.

1.6 Scope of this thesis

A survey of the current literature on protein folding indicates that well characterized cases mostly are small, monomeric, globular and single domain proteins which exhibit cooperative, reversible denaturation transitions under widely different physico-chemical conditions e.g. RNase A, HEWL, myoglobin, etc. On the other hand, large proteins often exhibit complex transition profiles, that are rarely reversible. The problem is further compounded by the fact that large proteins are often composed of several domains or even different polypeptide chains (oligomers).

To verify whether the rules for protein folding deduced from studies on small proteins are universal, it is imperative that folding be examined as a function over a wide range of protein size. The subject of investigations in this study is a large (N_{res}=370), monomeric, two-domain protein, MBP (Figure 1.1). Thermodynamic aspects of MBP folding are described in chapter2. Chapter 3 deals with general protein thermodynamics and describes how the size of a protein influences its folding. Mutations in an unstructured region of a polypeptide, affecting its thermodynamic and kinetic properties (as exemplified by MBP) is the focus of chapter 4. Chapter 5 discusses the unique aspects of MBP folding kinetics, influence of the chaperone SecB on the pathway of MBP folding and its implications. Chapter 6 is devoted to some aspects of the precursor form of MBP, preMBP. A summary of the main conclusions of this study is provided below.

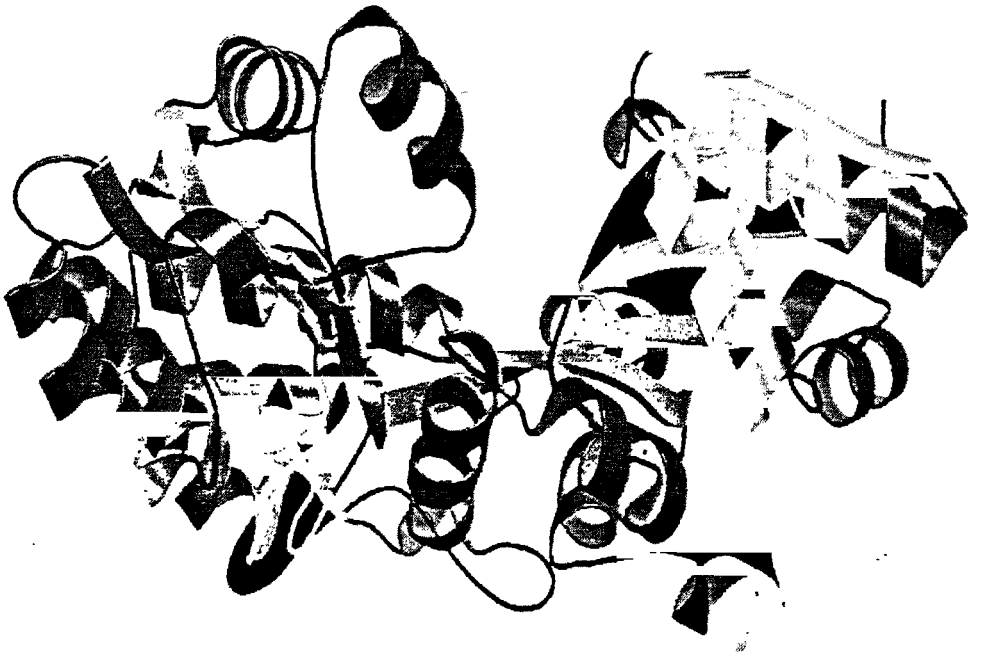

Figure 1.1. Molscript rendering of the structure of mature MBP, using the x-ray crystal structure coordinates (1omp.pdb). Maltose binds in the cleft inbetween the two domains.

Conclusions of work embodied in this thesis:

1) Size (of a protein) influences its folding and stability.

2) Slow phase of protein folding is not necessarily due to proline isomerization.

3) Protein precipitation is reversible in the case of MBP and MBP probably undergoes initial hydrophobic collapse during folding.

4) SecB promotes disaggregation of MBP.

5) The signal sequence affects both the thermodynamics as well as the kinetics of MBP folding.

6) MBP folding thermodynamics exhibits interesting qualitative differences from those of smaller proteins.

Avenues for further research :

1) the basis for the high degree of domain interaction and cooperativity of MBP folding

2) are the two domains in MBP formed separately or simultaneously and/or independently during folding ?

3) what residues are involved in the complex pH transitions?

4) structural analyses of the pH 3 molten globule form and the kinetic intermediates (x-ray scattering, time correlated single photon counting of bound ANS fluorescence to measure rotational correlation times, FT-IR spectroscopy etc.)

5) structure of cmMBP

6) structure of preMBP

7) the roles of periplasmic chaperones in protein folding in the bacterial periplasm